REMARKS

Status of the claims:

With the above amendments, claims 1-39 are pending with claims 11-16, 23, 24, 27, 31, 32, 34-37 being withdrawn from consideration from a prior restriction requirement. Claim 39 has been added and claims 1-10, 17-22, 25-26, 28-30, 33, and 38-39 are ready for further action on the merits. No new matter has been incorporated. Support for the change to the written description appears on page 41, line 8. New claim 39 has support on page 11, line 26 to page 12, line 16 of the written description.

All Pending Claims

For the Examiner's convenience all claims not withdrawn from consideration are presented here.

1. (Amended) A microorganism transformed with at least one recombinant DNA molecule encoding or causing the expression of a ~~gene~~ of at least one enzyme, that causes ~~the~~ functional coupling ~~of~~ the oxidation and reduction of substrates by two pyridine nucleotide-linked dehydrogenase reactions that share a common substrate and have different specificities for the NAD/NADH and NADP/NADPH coenzyme couples and so facilitates the transfer of electrons between the two coenzyme couples through the said

substrates, said transformed microorganism thereby producing an industrial product from carbohydrate more efficiently than does a corresponding non-transformed microorganism, said industrial product being more reduced than pyruvate.

2. (Amended) The microorganism of claim 1, said microorganism producing more product per unit of carbohydrate in a raw material than does a corresponding non-transformed microorganism.

3. The microorganism of claim 1, said microorganism producing a product faster than does a corresponding non-transformed microorganism.

4. The microorganism of claim 1, said microorganism producing less CO_2 per unit of a product produced than does a corresponding non-transformed microorganism.

5. The microorganism of claim 1, said microorganism having a reduced oxygen requirement per unit of a product produced than has a corresponding non-transformed microorganism.

6. (Amended) The microorganism of claim 1 that under the conditions of a biotechnological process, producing from

carbohydrates one or more industrial products more reduced than pyruvate, maintains a higher level of the metabolic capacity required to convert carbohydrate into said products in said process than does a corresponding non-transformed microorganism.

7. (Amended) The microorganism of claim 6, wherein the metabolic capacity required for the said process of a corresponding non-transformed microorganism decreases with time under the conditions of the said biotechnological process.

8. The microorganism of claim 1, wherein the product is ethanol.

9. The microorganism of claim 8, wherein the ethanol is derived from a pentose.

10. The microorganism of claim 8, wherein the ethanol is derived from a hexose.

17. (Amended) The microorganism of claim 1, wherein at least one of the recombinant DNA molecules encodes or causes the expression of a gene encoding an enzyme which is a pyridine nucleotide-linked dehydrogenase.

18. The microorganism of claim 17, wherein the dehydrogenase is selected from the group consisting of glutamate dehydrogenases, malate dehydrogenases, malic enzymes and aldehyde dehydrogenases.

19. The microorganism of claim 1, which microorganism is a yeast.

20. The microorganism of claim 19, which microorganism is a strain of *Saccharomyces* spp., *Schizosaccharomyces* spp. or *Pichia* spp.

21. (Amended) A microorganism of claim 9, which is a strain of *Saccharomyces* spp. or *Schizosacharomyces* spp. expressing genes encoding xylose reductase and xylitol dehydrogenase, and which is transformed with at least one recombinant DNA molecule encoding or causing the expression of a gene encoding an enzyme which is a pyridine nucleotide-linked dehydrogenase.

22. The microorganism of claim 21, which further expresses a gene encoding xylulokinase.

25. *Saccharomyces cerevisiae* strains selected from the group consisting of H1791 (VTT C-98298, DSM 12213), H1795 (VTT

C-98300, DSM 12214), H1803 (VTT C-98302, DSM 12215), H2193 (VTT C-99317, DSM 12722), H2195 (VTT C-99320, DSM 12723) and H2222 (VTT C-99322, DSM 12724).

26. *Schizosaccharomyces pombe* strains selected from the group consisting of H2369 (VTT C-99323, DSM 12725) and H2370 (VTT C-99324, DSM 12726).

28. (Amended) A method of producing useful products from carbohydrates, comprising the step of fermenting said materials with a microorganism of claim 1.

29. (Amended) The method of claim 28, wherein the carbohydrates comprise pentoses, pentose polymers or mixtures thereof.

30. (Amended) The method of claim 28, wherein the carbohydrates comprise hexoses, hexose polymers or mixtures thereof.

33. The method of claim 28, wherein ethanol is produced.

38. (Amended) A method of producing ethanol from carbohydrates comprising pentoses, pentose polymers or mixtures

thereof, comprising the step of fermenting said materials with a microorganism of claim 19.

Objections

The Examiner has objected to the language "recombinant DNA molecule encoding or otherwise causing the expression of". Applicants traverse this objection.

The language "recombinant DNA molecule encoding or otherwise causing the expression of at least one enzyme", has been amended in claims 1, 17 and 21 to recite the more accurate terminology "causing the expression of a gene of at least one enzyme". This phrase has support, for example, on page 13, line 20. Applicants have also deleted the word "otherwise" to more particularly claim the invention. No amendment has been made to the written description regarding this objection.

The Examiner has also objected to there being a conflict between the control strain of yeast. On page 42, in Table 3, the caption referred to the yeast strain "S. cerevisiae H2189" yet in the table the strain was referred to as "S. cerevisiae H2186". Table 3 has been corrected to correctly recite "S. cerevisiae H2189". Withdrawal of the objection is respectfully requested.

Rejections under 35 USC §112, second paragraph

Claims 1-10, 17-22, 28-30, 33 and 38 have been rejected under 35 USC §112, second paragraph as being indefinite. Claims 1, 17 and 21 have been rejected for reciting the following.

Claim 1 is rejected for reciting "at least one recombinant DNA molecule encoding or otherwise causing the expression of at least one enzyme". This rejection is traversed for the following reasons.

The language "recombinant DNA molecule encoding or otherwise causing the expression of at least one enzyme" in claims 1, 17 and 21 has been amended to recite the more accurate phrase "causing the expression of a gene at least one enzyme". This phrase has support e.g. on page 13, line 20. It is believed that with this language, these claims are no longer vague nor indefinite. Withdrawal of the rejection is respectfully requested.

Claim 1 is rejected for reciting "two pyrimidine nucleotide-linked dehydrogenase reactions with different specificities for the NAD/NADH and NADP/NADPH coenzyme couples." This rejection is traversed for the following reasons.

Applicants submit that "different specificities" is a more accurate description of the invention than "opposite specificities". There is support for this in the Summary of

the Invention, p. 6, lines 24 and 34, and again p. 10, line 33 and p. 21, line 17, etc. If one enzyme were ABSOLUTELY specific for NAD/NADH, then "opposite specificity" would be an ABSOLUTE specificity for NADP/NADPH. However, in practice, there is always a finite activity (maybe less than 1 %, maybe less than 1 in a million) with the other coenzyme couple. Thus, "opposite" could be interpreted to mean that this (very) minor activity with the other coenzyme was reproduced exactly, which is unlikely, not required, and hard to prove or disprove.

To further clarify the invention as described above, claim 1 has been amended to recite "... that share a common substrate and have different specificities" (see page 10, lines 32-33). Additional support can be found on pages 10 to 14 in general, particularly the equations (1 & 2), (3-5) and (3'-5'). On page 12, lines 26-27 it is written: "In reactions (3) to (5) the reduced substrate, SH_2 is common to the two dehydrogenases". Applicants submit that with this change and the above explanation, this rejection is obviated. Withdrawal of the rejection is respectfully requested.

Claim 1 has also been rejected for the phrase "so facilitates the transfer of electrons". This rejection is strenuously traversed for the following reasons. Applicants submit that there is nothing vague or indefinite about the use of this phrase. Applicants submit that one of skill in the art

[redacted]

would understand that the activation barrier is lowered so that transfer of electrons proceeds more easily.

The wording "facilitates the transfer of electrons", has support in the Summary of the Invention (p.6, line 30). Applicants submit that the Examiner's suggestion "equilibrates the transfer of electrons" is confusing, and Applicants fail to see how the Examiner's example (p. 11, line 28) supports this wording. The word "equilibrates" is not used in the specification in this respect, and when "equilibrate" does appear, it is used in the phrase "tends to equilibrate" (e.g., p. 10, lines 8 and 21). "Equilibrates" means "brings to equilibrium" and it would be hard to show this has happened, and Applicants submit it is not a requirement of the invention. Applicants submit that it is enough that the transfer of electrons between the two coenzyme systems is allowed to occur, i.e. facilitated. Accordingly, Applicants believe that the rejection has been obviated. Withdrawal of the rejection is warranted and respectfully requested.

The Examiner has also rejected claim 1 for the use of the phrase "useful products". This rejection is traversed for the following reasons.

The phrase "useful products" has been amended in the claims to recite " an industrial product . . . said industrial product being more reduced than pyruvate." Support for this

phrase is found in the paragraph bridging pages 3 and 4. In specific, p. 4 line 2 states: " . . . a central intermediate (such as pyruvate) that is more oxidised than the carbon source and reduce this intermediate to the desired product". Applicants thus believe that this makes the claim definite such that one of skill in the art would readily understand what is meant by this phrase. The desired product is therefore more reduced than the central intermediate (such as pyruvate). Accordingly, withdrawal of the rejection is respectfully requested.

The Examiner has also rejected claims 2 and 28 for the use of the phrase "raw material(s)". The phrase "raw material" has been amended to the word "carbohydrate". Corresponding amendments have been made to claims 1, 2, 6, 28-30 and 38. Support for the word "carbohydrate" as a "raw material" is found throughout the specification, for example on page 7, line 5. One of skill in the art would readily understand what is meant by "carbohydrate". Accordingly, withdrawal of the rejection is respectfully requested.

The Examiner has also rejected claim 6 for the use of the phrase "biotechnological processes". This rejection is traversed for the following reasons. The Examiner's attention is drawn to page 7, lines 5-8 where there are several Examples of "biotechnological processes". Accordingly, Applicants submit

that one of skill would understand precisely what is meant by "biotechnological processes", particularly when read in light of the specification. Withdrawal of the rejection is respectfully requested.

The Examiner has also rejected claim 7 for the use of the phrase "required metabolic capacity". This rejection is traversed for the following reasons. The expression "metabolic capacity" is qualified by "required" because Applicants do not mean here metabolic capacity in general but that required by the organism to carry out the biotechnological process of interest. Support for this phrase is found from page 22, line 24 to page 23, line 21, where it is pointed out that a significant parameter in a biotechnological process is the ability of the production organism to maintain not just its biomass (which may also be hard to measure), but also its metabolic capacity "to perform a particular set of biotransformations" (see page 23, line 19). Thus, on pages 22 to 23 the term "required metabolic capacity" is explained. Accordingly, when read in light of the specification, "required metabolic capacity" is neither vague nor indefinite. Withdrawal of the rejection is respectfully requested.

Rejections under 35 USC §112, first paragraph

[redacted]

Claims 1, 17 and 18 have been rejected under 35 USC §112, first paragraph, as lacking complete enablement. The Examiner asserts that while the specification is enabled for glutamate dehydrogenase and malic enzyme from S. cerivisiae, it does not reasonably provide enablement for any enzyme that is a pyridine nucleotide linked dehydrogenase or any malate dehydrogenase or aldehyde dehydrogenase. The Examiner further asserts that obtaining transformants with the claimed characteristics is unpredictable and is not routine. This rejection is traversed for the following reasons.

Applicants are not claiming all dehydrogenases or teaching that any dehydrogenase can be used to practice this invention. Claim 1 claims an enzyme that "causes the functional coupling of the oxidation and reduction of substrates by two pyridine nucleotide-linked dehydrogenase reactions that share a common substrate and have different specificities for NAD/NADH and NADP/NADPH". This is a smaller group of enzymes, not "essentially infinite" as alleged by the Examiner. The disclosure makes it clear that the invention can be practiced with pairs of dehydrogenases that have common substrates (reactions (1) and (2) on page 10) or at least one common substrate (SH_2 in reactions (3), (4) and (5) on pages 11 and 12 and S in reactions (3') to (5')). One skilled in the art would readily ascertain that longer cyclic reactions (e.g. (3") to

(5")) achieve the same objective as do the shorter reactions (3)-(5). However, Applicants have now limited claim 1 to "pairs of dehydrogenase reactions that share a common substrate". Further, on page 11, Applicants disclose several pairs of dehydrogenases that can be used to practice the invention and teach that the cofactor specificities of natural dehydrogenases can be changed to give man-made pairs of dehydrogenases with which to practice the invention. An example is given (Chen et al).

Finally, one of skill in the art would recognize that in an organism that contains malic dehydrogenase and malic enzyme in the same compartment, the instant invention can be practiced by adding pyruvate carboxylase (if this is not already present).

Accordingly, Applicants submit that one of skill in the art could make and use the invention commensurate in scope with the instantly claimed invention without undue experimentation. Withdrawal of the rejection is warranted and respectfully requested.

Rejections under 35 USC §102

Claim 1 has been rejected under 35 USC §102(b) as being anticipated by Boles (Boles et al., Eur. J. Biochem., 217, pp. 469-477, (1993).

Claim 1 has been amended so that the transformant disclosed in Boles no longer falls within the scope of the instantly claimed invention. In particular, the transformant taught by Boles can only survive on glucose when it contains functional mitochondria. Further, the transformant of Boles is unable to convert glucose into ethanol. See the last paragraph of Background of the Invention in the written description for a description of the Boles reference (p. 6, lines 5-21).

More particularly, the last paragraph of the Background (p. 6, lines 5-21 and especially lines 17 to 21) explains that Boles' yeast "converts NAD plus NADPH into NADH plus NADP, which is the opposite transformation to that required of the industrial production microorganisms". Boles may possess the enzyme equipment required to carry out the conversion required of the industrial organisms but these microorganisms lack phosphoglucoisomerase (the enzyme encoded by PGI).

Thus, when growing the microorganisms on hexoses, the microorganisms cannot use the normal glycolytic pathway but must pass all their carbons through glucose-6 phosphate dehydrogenase, thereby producing an excess of NADPH (not NADP, as in the industrial production microorganism of the instant invention). In order to live, the microorganisms must re-oxidize this NADPH, which the microorganisms do by converting it into

NADH (using the coupled GDH reactions) and when oxidizing the NADH with molecular oxygen in their mitochondria.

Therefore, the microorganisms "ability to survive on glucose was strictly dependent on the presence of functional mitochondria and oxygen and they were unable to convert sugars into ethanol" (see p. 6, lines 19 to 21). This sharply distinguishes the microorganisms of Boles from the microorganism of Claim 1, which produces industrial products more reduced than pyruvate, (in particular ethanol - see claim 8). One of the advantages of the present invention (p. 19, line 19; p. 22, tines 31 to 33 and claim 5) is that it decreases or eliminates the microorganism's requirement for oxygen. In contrast, Boles' yeast is completely dependent upon oxygen. Thus, Boles cannot anticipate the instant invention because it does not teach the elements of the instant invention. Withdrawal of the rejection is warranted and respectfully requested.

With the above remarks and amendments, it is believed that the claims, as they now stand, define patentable subject matter such that a passage of the instant invention to allowance is warranted. A Notice to that effect is earnestly solicited.

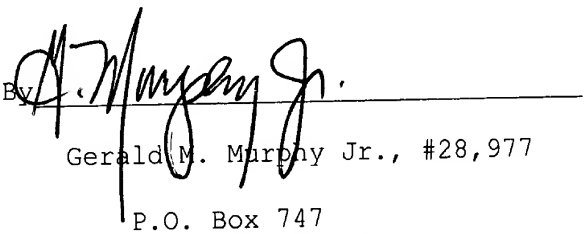
Pursuant to 37 C.F.R. §§ 1.17 and 1.136(a), Applicant(s) respectfully petition(s) for a one (1) month extension of time for filing a reply in connection with the present application, and the required fee of $110.00 is attached hereto.

If any questions remain regarding the above matters, please contact Applicant's representative, Gerald M. Murphy Jr., in the Washington metropolitan area at the phone number listed below.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. §§ 1.16 or 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By 

Gerald M. Murphy Jr., #28,977

GMM/TBS/crt

P.O. Box 747
Falls Church, VA 22040-0747
(703) 205-8000

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE WRITTEN DESCRIPTION:

The following changes have been made to the written description.

The caption to Table 3 and Table 3 on page 42 has been amended as follows.

--**Table 3.** Glucose fermentations with *Saccharomyces cerevisiae* recombinant strain expressing *MAE1* (H2193) and control strain (H2189): average fluxes expressed in either volumetric (J_V, C-mmol/l h) or specific (J_S, C-mmol/g-cell h) terms (time interval: 3.3 to 29 hours). Glucose and ethanol concentrations represent average values from four measurements: two with HPLC and two with enzymatic assays.

	Biomass (g/l)	Glucose (g/l)	Ethanol (g/l)
	H2193 H218[6]9	H2193 H218[6]9	H2193 H218[6]9
J_V (C-mmol/l h)	1.68 3.01	30.61 36.32	15.27 17.35
J_S (C-mmol/g-cell h)	2.60 3.27	47.37 39.47	23.64 18.85

IN THE CLAIMS:

The claims have been amended as follows.

1. (Amended) A microorganism transformed with at least one recombinant DNA molecule encoding or [otherwise] causing the expression of a gene of at least one enzyme that causes the functional coupling of the oxidation and reduction of substrates by two pyridine nucleotide-linked dehydrogenase reactions that share a common substrate and have [with] different specificities for the NAD/NADH and NADP/NADPH coenzyme couples and so facilitates the transfer of electrons between the two coenzyme couples through the said substrates, said transformed microorganism thereby producing an industrial product from carbohydrate [useful products] more efficiently than does a corresponding non-transformed microorganism, said industrial product being more reduced than pyruvate.

2. (Amended) The microorganism of claim 1, said microorganism producing more product per unit of carbohydrate in a raw material than does a corresponding non-transformed microorganism.

6. (Amended) The microorganism of claim 1 that under the conditions of a biotechnological process, producing from carbohydrates one or more industrial products more reduced than

[redacted]

pyruvate, maintains a higher level of the metabolic capacity required to convert carbohydrate into said products in [for the] said process than does a corresponding non-transformed microorganism.

7. (Amended) The microorganism of claim 6, wherein the [required] metabolic capacity required for the said process of a corresponding non-transformed microorganism decreases with time under the conditions of the said biotechnological process.

17. (Amended) The microorganism of claim 1, wherein at least one of the recombinant DNA molecules encodes or [otherwise] causes the expression of a gene encoding an enzyme which is a pyridine nucleotide-linked dehydrogenase.

21. (Amended) A microorganism of claim 9, which is a strain of *Saccharomyces* spp. or *Schizosacharomyces* spp. expressing genes encoding xylose reductase and xylitol dehydrogenase, and which is transformed with at least one recombinant DNA molecule encoding or [otherwise] causing the expression of a gene encoding an enzyme which is a pyridine nucleotide-linked dehydrogenase.

28. (Amended) A method of producing useful products from carbohydrates [raw materials], comprising the step of fermenting said materials with a microorganism of claim 1.

29. (Amended) The method of claim 28, wherein the carbohydrates [raw materials] comprise pentoses, pentose polymers or mixtures thereof.

30. (Amended) The method of claim 28, wherein the carbohydrates [raw materials] comprise hexoses, hexose polymers or mixtures thereof.

38. (Amended) A method of producing ethanol from carbohydrates [raw materials] comprising pentoses, pentose polymers or mixtures thereof, comprising the step of fermenting said materials with a microorganism of claim 19.

Claim 39 has been added.